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# ON THE ECOLOGY OF NOCARDIOFORM INTESTINAL ACTINOMYCETES OF MILLIPEDES (DIPLOPODA)

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Actinomycetes are often characteristic but in many respect less or completely unknown members of both aerobic and anaerobic indigenous gut microfloras of arthropods, worms etc. (Hungate 1946; Vályi-Nagy et al.1954; Szabó and Marton 1966; Szabó et al.1967, 1976; Szabó 1974; Bignell et al. 1980; Bignell 1984, etc.).

Some years ago we revealed (Dzingov et al. 1982; Szabó et al. 1983) that yellow coloured smearable bacteroid nocardioforms of oxidative and partly also fermentative metabolism can attain large population sizes in the gut of millipedes. On studying them and describing their first species as Promicromonospora enterophila (Jáger et al. 1983; Szabó et al. 1985) it became obvious that they are very closely related to certain, from ecological point of view almost unknown genera and groups of actinomycetes such as Oerskovia, Promicromonospora nonmotile Oerskovia-like-(NMOs; Lechevalier and Lechevalier 1981) and other related organisms, isolated randomly, presumably as disseminating propagules, from laboratory- and clinical-materials, soils, etc. (Krassilnikov et al. 1961; Lechevalier 1972; Sottnek et al. 1977; Yevtushenko et al. 1982, etc.). Accordingly, dealing with such intestinal partners of millipedes we can contribute to our knowledge also on the biology of the above-mentioned nocardioform genera and groups, which together with this gut actinomycetes will be designated below simply as OPNs (Oerskovia-Promicromonospora-NMO-type microbes).

First of all, we touch upon the definition of "nocardioforms", then outline the millipede gut as an environment for them.

## NOCARDIOFORM ORGANISMS: A VERY HETEROGENEOUS GROUP OF ACTINOMYCETES

Nocardioforms (Prauser 1967) are intermediates between coryneform bacteria and sporoactinomycetes (Prauser 1981), which reproduce only by fragmentation of their hyphae. It might be supposed that among them Promicromonospora is a minor intermediate towards sporoactinomycetes, and Cellulomonas

connects them with coryneforms. This supposition could, however, be confirmed only with difficulty because both coryneforms (Goodfellow and Minnikin 1981a; Goodfellow et al. 1982; Seiler 1983) and nocardioforms (Goodfellow and Minnikin 1981b) are very heterogeneous groups comprising different genera. Besides, according to the results of numerical phenetic surveys, analyses of DNA bases, cell walls, lipids, etc. it seems to be more reasonable to study these organisms on the basis of new groupings, not respecting the earlier proposed nocardioform, coryneform, etc. distinctions (Goodfellow and Minnikin, 1981a).

Without doubt, the taxonomic inconsistencies may considerably be responsible for our limited knowledge also about their ecology. At present the correct diagnostic descriptions on invertebrate gut nocardioform isolates seem to be more important than their taxonomic identification using the nomenclature of one of the proposed systems.

## THE MILLIPEDE GUT AS AN ENVIRONMENT FOR OPNs

The main characteristics of the forest litter consuming millipedes' gut environment are:

a, The foregut is only poorly utilized by microorganisms.

b, The midgut represents the absorptive surface and continuously secretes the semipermeable peritrophic membrane which is perhaps a barrier to microorganisms. This encloses the solid food materials - preventing their direct contact with the epithelium - into the endoperitrophic space where the enzymatic degradation of macromolecules is taking place, while the hydrolysis of dimers and oligomers proceeds in the ectoperitrophic space.

c, The cuticle-lined hindgut is strongly developed and bears both flat cuticular surfaces and ornaments such as spines of various shapes, which assist in shredding the peritrophic membrane and provides sites for microbial colonization. It contains a rich and complex flora: common enterobacteria (Klebsiella etc.), nocardioform actinomycetes, selected soil bacteria (Micrococcus, Bacillus etc.), unknown filamentous procaryotes and presumedly eucaryotes observed otherwise already more than 130 years ago (Leidy 1850) and others.

d, Malpighian tubules generate a fluid containing a wide range of organic and inorganic anions, nitrogenous compounds, etc. (Maddrell 1981), mostly easily utilizable by nocardioforms. These secreted materials and the fluid input to the gut via salivary glands (containing mucous components and digestive enzymes) provide suitable environment for intense multiplication of many other microbes too.

e, In the millipede gut conditions are far from anaerobic.E.g. the redox potencials ($E_h$) vary in the midgut of Glomeris marginata between +267, and +307 mV and in its hindgut between +167 and +277 mV (Bignell 1984). Accordingly,

in this gut environment also obligate aerobic nocardioforms may be good competitors with bacteria.

## OCCURRENCE OF OPNs IN THE FREE SOIL MATRIX

Regarding the data of Soviet and American workers (Lechevalier and Lechevalier 1981; Yevtushenko et al. 1982, etc.), OPNs may be ubiquitous organisms widely distributed in soils all over the world. The occurence of them was detected by us also in Hungarian soils. But in our opinion they do not belong to the true indigenous terrestrial microbes residing in the free soil matrix.

It might be supposed that there are particular genetic soil types, varieties or at least soil horizons, subhorizons, microhabitats, etc. preferred and colonized by them, but such milieus have not been revealed so far. Also in samples of litter layers ($A_{oo}$-$A_o$) and $A_F$-horizon matter of brown forest soils where millipedes occur and feed in relatively dense populations and lay their excrements, OPNs are present only sporadically or not at all.

E.g. from a composite sample of $A_F$-horizon matter rich in old millipede faecal pellets collected in the feeding habitat of different millipede species, among them Chromatoiulus projectus, in November when the feeding activities of them reduced already strongly, altogether 1523 isolates were obtained in the following distribution: Bacillus cereus-type isolates 118; Bacillus subtilis-type isolates 26; green fluorescent bacteria 79; a heterogeneous group of different bacterial isolates 640; Streptomyces spp about 17 types or species 267; sterile actinomycetes (their hyphae did not show fragmentation) 371; other not OPN-type actinomycetes 22; OPNs 0.

Another composite $A_F$-horizon sample obtained from a Chromatoiulus-nest in a more favourable time point was predominated by Streptomyces spp (more than 10 species), members of other actinomycete genera (more than 6 species) and many types of bacteria, contained OPNs in less than two per cent of all isolates.

At present it could be said that the probability of finding OPNs in a soil sample is increasing with the amount of fresh millipede (or other, OPNs-bearing invertebrate-) faecal matter incorporated in it.

## OCCURRENCE OF OPNs ON THE EXOSKELETON OF MILLIPEDES

Millipedes play an important role as animal vectors in the passive dispersal of soil microbes (Szabó et al. 1983) transporting them, mostly adhered to the projections of the tarsal region of legs, and inoculating with them the freshly exponed, still uncolonized dead organic matter surfaces present in the soil. According to the results of our electron-microscopic observations on different regions of the millipede's exoskeleton and bacteriological analyses on the leg

surface-flora, OPNs do not belong to the external partners of these animals at all. The overwhelming majority of the leg--isolates proved to be Streptomyces spp (more than 17 by legs transported species were studied by us; Dzingov et al. 1982; Szabó et al. 1983). The strongly hydrophobic negatively charged streptomycete propagules (see Ruddick and Williams 1972) are attracted in large numbers by the hydrophobic cuticle of the millipede's exoskeleton, from which OPNs were not isolated.

## OCCURRENCE OF OPNs IN THE GUT OF DIFFERENT SOIL INVERTEBRATES

Promicromonospora enterophila proved to be a stable and only in its population densities varying member of the hindgut-community of Chromatoiulus projectus. OPNs (physiologically more or less different of P.enterophila; Márialigeti and Contreras, unpublished) regularly occur also in the gut of other Diplopoda such as Leptoiulus proximus, Cylindroiulus luridus, C.boleti, Unciger foetidus etc., as it was revealed in our Department. Fernandez isolated (unpublished) a lot of Promicromonospora-type strains from the hind gut of the millipede Polydesmus complanatus.

From the intestine of specimens of Protracheoniscus amoenus (Isopoda) OPNs are missing completely, although this woodlice feeds frequently together with Ch.projectus on fallen leaves of the same trees in very same litter-environments where they can presumedly contaminate each other with their faecal bacteria (Márialigeti et al. 1984).

A detailed numerical phenetic survey carried out by Jáger in this year on the platable microflora of fresh faecal matter of the springtail Tomocerus longicornis (Collembola) schowed that OPNs are lacking also in this soil animal belonging similarly to the forest litter biota.

OPNs were not found in the gut contents of adult specimens of the earthworms Eisenia lucens, Octolasium montanum, Fitzingeria platyura depressa, Allolobophora rosea and the juvenile specimens of Lumbricus polyphemus collected from the A-horizon of a brown forest soil either. But surprisingly, it has succeeded to show (Ravasz et al. 1985a,b) a large homogeneous, facultatively anaerobic Oerskovia-type OPN-population from the gut contents of adult specimens of the earthworm Lumbricus polyphemus. According to Ravasz, all of the selected and thoroughly studied 25 representative strains of this OPN-population proved to be identical with each other. As regards the stability of the partnership between these OPNs and Lumbricus polyphemus we have at present no data, but it does not seem very close.

Taking all of these findings into consideration, some important questions may be exposed: How large can be the host range of these nocardioform gut colonizers? Does it involve a few or many different taxonomic groups of invertebrates? Are these gut colonizers different at species, generic or even familial levels? Is the gut of specimens of the very same animal species colonized consistently by the same type of nocardioforms or not? etc.

As regards the first question, our experiences have lead us to believe that their host range is very large. OPNs adapted themselves, during their evolutionary history, not exclusively to millipedes at all. In this respect it might be interesting to note that in the past many workers detected in the faeces of different invertebrates not only Corynebacterium (as e.g. Baleaux and Vivares in the case of the millipede Schizophyllum sabulosum var. rubripes, in 1974), but also simply as coryneform bacteria which in several cases might be nocardioforms in accordance with OPNs. Khambata and Bhat (1955) e.g. reported on the occurrence in tropical earthworms of yellow coloured, doubtfully motile, short rods and filaments partly with swollen end called coryneform bacteria.

As regards the questions on the systematic relationship of these gut nocardioforms, a pregnant answer was given by the results obtained on studying (Chu Thi Loc 1985) the gut-OPNs of the millipede Glomeris hexasticha.

## THE NOCARDIOFORM GUT-PARTNERS OF CHROMATOIULUS PROJECTUS AND GLOMERIS HEXASTICHA

Many problems have arisen already at the identification (Jáger et al. 1983) of P.enterophila, the gut-partner of Ch.projectus. Its identity at generic level with Promicromonospora was questioned by some workers suggesting that it belongs perhaps to a new genus. On the other hand, Prauser /personal communication/ showed that its type strain is sensible to a highly specific Oerskovia-phage. Consequently, it might belong to oerskoviae, so much the more because it also has fermentative abilities. Also the adequacy of the name Promicromonospora which was introduced by Krassilnikov et al. in 1961, as P.citrea, for some randomly isolated identical strains is questionable if we are considering the results of Kuimova et al. (1983), according to which in P.citrea spore formation, similar to that in Micromonospora, it does not exist at all.

In 1980 Anderson and Bignell supposed that in the faeces of the millipede Glomeris marginata multiply rather soil bacteria than the specific gut symbionts. But what kind of gut symbionts? In the hindgut of this animal Professor Anderson has observed with the aid of electronmicroscope among others unidentified monosporic actinomycetes (personal communication, 1982).

In 1983 in our Department Chu Thi Loc isolated such microbes from fresh faeces of another Glomeris species G.hexasticha, common in Hungary. These behaved as it was supposed by Anderson and Bignell (1980) and as P.enterophila. They originated from the hindgut, quickly dissappeared from the faeces and occurred in the soil sporadically at most. On their identification many at present hardly resolvable problems have arised.

On the first approach, the selected representative strains, which were identical with each other showing that also G.hexasticha possesses its own homogeneous nocardioform

gut population, seemed to be the members of Promicromonospora. Their growth and multiplication have proceed according to a very complex life cycle including all morphological elements which were revealed in P.citrea by Kuimova et al. (1983): rod and coccus shaped vegetative cells or hyphal bits, chlamydospores, oval or sphaerical spore-like lateral bodies, swollen vesicles, subterminal vesicular bodies, unseptated extensively branched substrate hyphae which rapidly broke up into fragments, etc. Also the biochemical characteristics of these obligately aerobic strains showed a very close similarity to those of P.citrea. But in the cultures of the Glomeris-strains a few actively moving flagellated cells were also observed among the many others showing only Brownian movement. This was the first contradiction to the authentic description of Promicromonospora, but not in general to the summarized characters of OPNs.

Our nocardioform Glomeris-strains proved to be photochromogenic organisms: their colonies became yellow only after 1-2 days cultivation on agar media in illuminated surroundings. They produced besides also diffusible red-coloured pigments in certain agar-media. These properties, however, are completely lacking in any one true Promicromonospora species or strains. In contranst to the strains of P.enterophila, the Glomeris--nocardioforms did not show any sensibility against Oerskovia-,Promicromonospora-phages /Prof. Prauser, Jena: personal communication/. Finally, one of our coauthors, I.Szabó /Debrecen/ has detected differences in the major cell-wall constituents of strains of P.enterophila and those of Glomeris-nocardioforms.

At present it might be supposed that the individual invertebrate species, genera or larger taxa may harbour (if they have at all) their own nocardioform species, genera or groups, but still many further data would be necessary to confirm this supposition.

## SURVIVAL CAPABILITY OF P.ENTEROPHILA IN SOIL

As we have repeatedly stated, OPNs disappear quickly from the laid faeces of their host animals and do not participate in the community metabolism of the soil biota. We did try to clarify the nature of environmental stresses responsible for this phenomenon.

In the framework of a series of laboratory experiments we compared the survival of P.enterophila in untreated and heat sterilized $A_F$-horizon material of an eutrophic brown forest soil. Soil samples in Erlenmayer flasks were inoculated with a standard cell-suspension of P.enterophila and incubated at 28°C for a year. Moisture content of the samples was about pF 2 and water loss was continuously replaced by sterile water. The presence of alive P.enterophila propagules in the samples (at least in two parallels) was detected at different time points by random

reisolations using the plate-count technique and nutrient-furthermore starch-agar as plating media.

Reisolations were carried out on six occasions. Altogether 1930 P.enterophila isolates were obtained from sterile soil samples, among them 1200 ones already after a whole year incubation. From the untreated soil samples, after two weeks incubation we obtained altogether 1773 isolates, but among them we did not find P.enterophila at all. This gut actinomycete disappeared in the presence of the complex soil microbiota extraordinarily quickly. The result of this experiment was identical if we used for inoculation of the samples a suspension containing P.enterophila in tenfold germ number. This nocardioform survived, however, in sterile soil samples a year without changing the growth activity of its reisolates. This experiment was later repeated using other forest soil matter for cultivation.

During the second series altogether 1488 isolates were obtained among them only 8 P.enterophila, from untreated soil samples of a water content of about pF 0 and 2, respectively. Also in this series P.enterophila dissappeared from the samples quickly i.e. within two months. Its elimination was more rapid at pF 2 than at pF 0, which provides less advantageous physical conditions for microbial activities.

The highest limit of the duration of its survival in sterile soil we could not be clarified, but we suppose that it can remain alive for a very long time. All these render probable that biotic factors may be responsible for the disappearance of this microbe from the soil.

## ARE THE PROPAGULES OF OPNs SUFFICIENTLY DURABLE STRUCTURES FOR AERIAL DISPERSAL?

Although Oerskovia has motile flagellated forms which can migrate actively by swimming, and this stage is presumedly implicated in its dispersal, OPNs are subjected more frequently to dissemination by wind and air. Freshly laid millipede pellets dry quickly up and disintegrate on the surfaces of fallen leaves in the litter layer where these animals regularly feed. From laboratory materials or from anywhere randomly isolated OPNs may derive from windblown litter or soil matter. Cells, bits of hyphae etc. of OPNs may adhere to minute dry excrement fragments, easily raised by wind and they may travel as airborne particles for long distances. But are their propagules sufficiently durable for such travels? Those of P.enterophila remained in our laboratory alive for more than six months in air-dry condition in sterile dry $A_F$-horizon matter-, quartz sand- and clay-samples, without decreasing their viability (which was checked on 968 reisolated cultures).

It is very likely that millipedes did not develope any particular mechanism for the transmission of their OPNs gut partners to their offsprings. The digestive canal of the latters may be contaminated by these microbes by oral uptake on feeding on organic particles bearing disseminating nocardioform propagules. We did not detect the presence of

P.enterophila within or on the surface of eggs laid by Ch.projectus into the soil at all.

## COMPETITIVENESS AND RESISTANCE TO SOIL MICROBES

After it became obvious that OPNs do not possess traits which would endow them with the capacity to cope with biological stresses of the free soil surroundings, it seemed to be interesting to evaluate the range of tolerance of P.enterophila against different biotic influences. Already on studying the influence of changing water conditions in non--sterile with P.enterophila inoculated soil samples checking the alteration in the composition of the microbiota on 1023 isolates it was found that at about pF 6, when owing to the low moisture content the microbial antagonistic activities strongly decrease, P.enterophila survived >10 months in large population sizes.

On the other hand, stimulating the activities of indigenous microorganisms in nonsterile with P.enterophila inoculated $A_F$-samples, at pF 2, with adding into them pepton, cellulose, starch, glucose or yeast extract, respectively, in 1 per cent concentrations or ammonium-phosphate in a tenth one, the disappearance of this nocardioform, in comparison with the rate of its elimination from the unamended control samples, was accelerated (to a few weeks or even less). This was checked by studying altogether 5041 isolates in the overwhelming majority soil bacteria and actinomycetes.

If the $A_F$-horizon matter was heat sterilized, but after this procedure reinoculated simultaneously with P.enterophila and a dense suspension in tap water of an alkali soil sample (solonchak) containing the complex original microflora the alien soil biota proved to be unable to destroy the nocardioform population exponed to it. After 9 months incubation (at pF 2) still 52 per cent of all isolates (382) belonged to P.enterophila. From non-sterile solonchak and acid peat soil-samples (pF 2) however, P.enterophila disappeared within two months.

In sterilized $A_F$-matter synchronously reinoculated with a suspension of $A_F$-material of the same origin, but heated only at 70°C for 10 minutes or with a suspension containing the complete original $A_F$ microbiota and P.enterophila, the latter was eliminated more slowly than in the untreated non-sterile $A_F$-samples.

Consequently, it can be concluded that P.enterophila can survive, or at least prolong its existence, in presence of a complex microflora only if the soil surroundings are alien and still uncolonized for/by the latter.

Another interesting finding was that in sterile samples of $A_F$-horizon matter (pF 2) the survival of P.enterophila was not limited, or at least not considerably, in mixed populations with a lot of soil microbes. Among those which were cocultivated with it there were also common soil bacteria and fungi possessing prominent community positions

under natural soil circumstances and also in the original $A_F$-samples used for these experiments.

During several months P.enterophila and a strain of Aspergillus niger showed in soil cocultures only slow changes in the ratio of their population densities checked by the aid of plate count estimates and reisolations. After 1 month incubation their ratio was in previously heat-sterilized $A_F$-matter 24:7, two ones 15:4, five ones 8:4, etc. Having cocultivated it under such conditions with a Penicillium sp. strain the results were similar.

With inoculation into sterile $A_F$-samples introduced, the density of population of P.enterophila practically did not change during a year incubation if these samples were simultaneonsly inoculated with one of three Streptomyces spp. strains (No 11/658, 15/107 and 22/181, respectively) or three Bacillus spp. ones (1/635, 2/858 and 5/123, respectively) which proved to be codominant organisms in the untreated $A_F$-samples. This statement was confirmed by studies on altogether 7900 reisolated cultures of them.

P.enterophila also maintained its approximative initial inoculation density during a year when we cultivated it in previously sterilized $A_F$-samples as a member of an artificially created community composed of the above-listed six soil strains. During this experiment, on checking the survival of this nocardioform, from this complex seven members population altogether 2640 reisolated cultures were obtained. At the end of the cocultivation, P.enterophila constituted still 29 per cent of the mixed population.

All these results indicate that biological factors may be responsible for the low rate of survival of P.enterophila in natural soils, and in this respect there exists a definite range of growing environmental stresses: sterile soil + pure or mixed bacterial-and actinomycete-populations < sterile soil + reinoculation with moderately heat treated soil suspension < sterile soil + reinoculation with untreated soil suspension < natural untreated soil.

It is also without doubt that P.enterophila possesses a certain grade of competitive ability which, however, is not enough to compete succesfully with the members of an established complex natural soil microbial population, and to replace or displace only a single one of them.

Finally, we observed that in sterile $A_F$-samples survival and viability of P.enterophila were not limited or influenced at all if these samples were wetted with sterile filtrates of suspensions or water-extracts of freshly collected $A_F$-horizon material.

## P.ENTEROPHILA AS A MEMBER OF THE MILLIPEDE'S HINDGUT COMMUNITY

Different interspecific metabolic relationships (on which no data are available) may exist among the members of in its composition less studied microbiota in the also

poorly researched chemical environment of the millipede's hindgut lumen.

Contreras (1985), who studied the species composition and biochemical properties of the hindgut flora of Ch.projectus by the aid of numerical phenetic analyses, has isolated and selected a lot of Klebsiella-, Pseudomonas-, Micrococcus- etc. strains representing the codominant platable gut bacteria of this millipede. Ten different strains of them were contrasted by Heydrich with nine P.enterophila strains on nutrient- and synthetic glucose-agar plates using the cross streaking method to clarify the frequency and types of their mutual interactions. Table 1 shows the number and distribution of antagonistic and stimulative interactions between them.

As can be seen, this gut microflora might generally be considered in the relation of these media (!) a neutral community characterized by low numbers of detectable interactions among its members also on synthetic agar. Strains of P.enterophila produce otherwise no antimicrobial substances acting in interspecific, -generic, etc. relations, as this was detected in several tests using Escherichia coli, Bacillus subtilis, other bacteria, yeasts, fungi, etc. as test organisms. This behaviour is very characteristic of many other strains of different species of OPNs studied by us so far. This neutrality observed among the representatives of the Ch.projectus hindgut flora was not copletely confirmed on contrasting bacterial strains isolated from the gut of specimens belonging to different millipede species. E.g. a Bacillus cereus strain (No 24) isolated from Cylindroiulus boleti antagonized some P.enterophila strains, and many other gut strains isolated both from Ch.projectus and C.boleti.

It is interesting to note that some P.enterophila strains proved to be able to antagonize slightly some other strains of their own species. Does perhaps any bacteriocinogenesis exist in the population of this species? At present we cannot give answer on this question as well as on many others concerning the ecology and biology of the gut flora of millipedes.

OPNs studied so far at our Department seem to be only moderately adapted to the animal's gut milieu. They are easily cultivable on laboratory media, have simple nutritional requirements and can multiply with inorganic N-sources and simple organic compounds such as glucose, arabinose, fructose, glycerol, lactose, raffinose, acetate, malate, succinate, etc. as sole source of carbon. They do not decompose cellulose and a lot of complex organic compounds, about which we suppose that are hardly available for their host animals. In our opinion, they multiply in the hind gut on the expense of simple hydrolytic products of polysaccharides, furthermore on proteins. It is possible that the host animals can utilize the cell-materials of OPNs. Such a phenomenon was showed by Reyes and Tiedje (1976) on studying the terrestrial isopod Tracheoniscus rathkei, which can intensively digest the resident gut microorganisms particularly under conditions of starvation.

Table 1. Number, distribution and types of mutual interactions among 9 P.enterophila- and 10 Pseudomonas-, Klebsiella-, Micrococcus- etc. strains representing the gut flora of Ch. projectus on nutrient- and synthetic glucose-agar plates

| | Nutrient-agar | | Synthetic glucose-agar | |
|---|---|---|---|---|
| | Total | % | Total | % |
| Number of contrastings | 361 | 100 | 361 | 100 |
| Detected antibiotic actions | 3 | 0,8 | 30 | 8,3 |
| Detected stimulations | 0 | 0 | 22 | 6,1 |
| Promicromonosporae among themselves contrastings | 81 | 100 | 81 | 100 |
| (antibiotic) interactions | 3 | 3,7 | 7 | 8,6 |
| stimulations | 0 | 0 | 0 | 0 |
| Promicromonosporae←→other bacteria contrastings | 180 | 100 | 180 | 100 |
| antibiotic interactions | 0 | 0 | 15 | 8,3 |
| stimulations | 0 | 0 | 14 | 7,7 |
| Bacterial strains among themselves contrastings | 100 | 100 | 100 | 100 |
| antibiotic interactions | 0 | 0 | 8 | 8 |
| stimulations | 0 | 0 | 8 | 8 |

## PERSPECTIVES

OPNs and closely related organisms are widely distributed in Nature. According to Williams and Wellington (1982), Oerskovia easily isolated from soil, but its frequency of ocurrence is still questionable. Prauser (1976) remarked that "The relative small number of strains of Nocardioides, Oerskovia and Promicromonospora isolated in different laboratories seems to be incompatible with the ease of getting the respective phage from soil". This dilemma does not seem to be insoluble if we are taking into consideration that they are not true soil microorganisms, but intestinal ones of soil invertebrates.

The yellow coloured, quickly fragmenting mycelia, chlamydospores, single monospore-like lateral structures,

swollen vesicles, subterminal vesicular bodies, nonmotile or flagellated cells producing oxidative or facultatively fermentative organisms of simple nutritional requirements characterized by type VI. or eventually other cell wall composition are perhaps the members of a less known and so far only sporadically studied large and variable taxon which now might provisionally be designated as a group of "enteroactinomycetes". The host-range of these organisms is presumably broad, involving very different taxonomic groups of invertebrate animals.

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